

What is claimed is:

1 1. A method for selecting cells based on whether the cells express a short-lived
2 protein, the method comprising:
3 taking a library of cells, each cell in the library expressing a fusion protein
4 comprising a reporter protein and a protein encoded by a sequence from a cDNA
5 library derived from a sample of cells, the sequence from the cDNA library varying
6 within the cell library;
7 modifying a rate of protein expression or degradation by cells in the library;
8 and
9 selecting a population of cells from the library of cells based on the
10 population of cells having different reporter signal intensities than other cells in the
11 library, the difference being indicative of the population of cells expressing shorter
12 lived fusion proteins than the fusion proteins expressed by the other cells in the
13 library.

1 2. A method according to claim 1 wherein the reporter protein is a fluorescent
2 protein.

1 3. A method according to claim 1 wherein the reporter protein is a green
2 fluorescence protein (GFP) or enhanced green fluorescence protein (EGFP).

1 4. A method according to claim 1 wherein protein expression is inhibited and
2 selecting a population of the cells is based on the selected population of cells having
3 a lower reporter signal intensity than the other cells after modifying the rate of
4 protein expression.

1 5. A method according to claim 1 wherein protein expression is inhibited and
2 selecting a population of the cells is based on the selected population of cells having
3 less than half the reporter signal intensity than the other cells after modifying the rate
4 of protein expression.

1 6. A method according to claim 1 wherein protein degradation is inhibited and
2 selecting a population of the cells is based on the selected population of cells having
3 a higher reporter signal intensity than the other cells after modifying the rate of
4 protein degradation.

1 7. A method according to claim 1 wherein protein degradation is inhibited and
2 selecting a population of the cells is based on the selected population of cells having
3 more than twice the reporter signal intensity than the other cells after modifying the
4 rate of protein degradation.

1 8. A method according to claim 1 wherein the selected population of the cells
2 are subjected to one or more additional rounds of selection, each round of selection
3 comprising modifying a rate of protein expression or degradation by the cells, and
4 selecting a further subpopulation of the cells based on whether the cells have
5 different reporter signal intensities than the other cells.

1 9. A method according to claim 1 wherein the selected population of the cells
2 are subjected to one or more additional rounds of selection such that at least one
3 round of selection comprises inhibiting protein expression and at least one round of
4 selection comprises inhibiting protein degradation.

1 10. A method according to claim 1 wherein the selected population of the cells
2 are further selected, at least partially, by culturing cells separately and individually
3 monitoring how the reporter signal of each cell culture changes in response to
4 protein synthesis or protein degradation being inhibited.

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1 11. A method according to claim 1 wherein the selected population of cells are
2 further selected, at least partially, by culturing cells separately and individually
3 monitoring how the reporter signal of each cell culture changes using a fluorescent
4 plate reader.

1 12. A method according to claim 1 wherein the method further comprises
2 analyzing whether the fusion protein of the selected cells is short-lived by a pulse-
3 chase analysis.

1 13. A method according to claim 1 wherein the method further comprises
2 analyzing whether the fusion protein of the selected cells is short-lived by
3 radiolabelling the expressed fusion protein;
4 immunoprecipitating the expressed fusion protein with anti-GFP antisera;
5 and
6 analyzing the immunoprecipitate by SDS-PAGE and autoradiography.

1 14. A method according to claim 1 wherein the method further comprises
2 determining the nucleic acid sequences of the fusion proteins of the selected cells.

1 15. A method according to claim 1 wherein the method further comprises
2 determining the protein sequences of the fusion proteins of the selected cells.

1 16. A method according to claim 1 wherein the method further comprises
2 analyzing whether a portion of the fusion protein encoded by the sequence from the
3 cDNA library is short-lived when expressed independent of the reporter protein.

1 17. A method for selecting cells based on whether the cells express a short-lived
2 protein, the method comprising:
3 taking a library of cells, the cells in the library expressing a first reporter
4 protein and a fusion protein comprising a second reporter protein and a protein
5 encoded by a sequence from a cDNA library derived from a sample of cells, the

sequence from the cDNA library varying within the cell library;
modifying a rate of protein expression or degradation by cells in the library;
and
selecting a population of cells from the library of cells based on the
population of cells having different normalized reporter signal intensities than other
cells in the library, the normalized reporter signal intensity comprising a reporter
signal from the fusion protein normalized relative to a reporter signal from the first
reporter protein, the difference being indicative of the population of cells expressing
shorter lived fusion proteins than the fusion proteins expressed by the other cells in
the library.

18. A method for selecting cells based on whether the cells express a short-lived
protein, the method comprising:

taking a library of cells, the cells in the library expressing a fusion protein
comprising a reporter protein and a protein encoded by a sequence from a cDNA
library derived from a sample of cells, the sequence from the cDNA library varying
within the cell library;

partitioning the library of cells into populations of cells based on an intensity
of a reporter signal from the fusion protein such that cells partitioned into a given
population have a reporter signal within a range of reporter signal intensity;

modifying a rate of protein expression or degradation by cells for a given
population of cells; and

selecting a subpopulation of cells from the given population of cells based on
the subpopulation of cells having different reporter signal intensities than other cells
in the given population, the difference being indicative of the subpopulation of cells
expressing shorter lived fusion proteins than the fusion proteins expressed by the
other cells in the given population.

19. A method according to claim 18 wherein the reporter protein is a fluorescent
protein and the range of reporter signal intensity is equal to or less than a half-log
interval of fluorescence.

1 20. A method according to claim 18 wherein the reporter protein is a fluorescent
2 protein and partitioning the screened cells into populations of cells comprises
3 partitioning the screened cells into populations such that a given population has a
4 modal brightness that differs from another population by a factor of at least 3.

1 21. A method according to claim 18 wherein partitioning the screened cells into
2 populations of cells comprises partitioning the screened cells into at least 4
3 populations of cells where the reporter signal intensities of cells within a given
4 population do not overlap with the reporter signal intensities of cells within another
5 population of cells.

1 22. A method according to claim 18 wherein protein expression is inhibited and
2 selecting a subpopulation of the cells is based on the subpopulation of cells having a
3 lower reporter signal intensity than the other cells after protein expression is
4 inhibited.

1 23. A method according to claim 18 wherein protein expression is inhibited and
2 selecting a subpopulation of the cells is based on the subpopulation of cells having
3 less than half reporter signal intensity than the other cells after protein expression is
4 inhibited.

1 24. A method according to claim 18 wherein protein degradation is inhibited and
2 selecting a subpopulation of the cells is based on the subpopulation of cells having a
3 higher reporter signal intensity than the other cells after protein degradation is
4 inhibited.

1 25. A method according to claim 18 wherein protein degradation is inhibited and
2 selecting a subpopulation of the cells is based on subpopulation of cells having more
3 than twice the reporter signal intensity than the other cells after protein degradation
4 is inhibited.

26. A method according to claim 18 wherein the selected subpopulation of the cells are subjected to one or more additional rounds of selection, each round of selection comprising modifying a rate of protein expression or degradation by the cells, and selecting a further subpopulation of the cells based on whether the cells have different reporter signal intensities than the other cells.

27. A method according to claim 18 wherein the selected subpopulation of the cells are subjected to one or more additional rounds of selection such that at least one round of selection comprises inhibiting protein expression and at least one round of selection comprises inhibiting protein degradation.

28. A method according to claim 18 wherein the selected subpopulation of cells are further selected, at least partially, by culturing cells separately and individually monitoring how the reporter signal of each cell culture changes in response to protein synthesis or protein degradation being inhibited.

29. A method according to claim 18 wherein the selected subpopulation of cells are further selected, at least partially, by culturing cells separately and individually monitoring how the reporter signal of each cell culture changes using a fluorescent plate reader.

30. A method according to claim 18 wherein the method further comprises determining the nucleic acid sequences of the fusion proteins of the selected subpopulation of cells.

31. A method according to claim 18 wherein the method further comprises determining the protein sequences of the fusion proteins of the selected subpopulation of cells.

32. A method for selecting cells based on whether the cells express a short-lived protein, the method comprising:

taking a library of cells, the cells in the library expressing a first reporter protein and a fusion protein comprising a second reporter protein and a protein encoded by a sequence from a cDNA library derived from a sample of cells, the sequence from the cDNA library varying within the cell library;

partitioning the library of cells into populations of cells based on an intensity of a reporter signal from the fusion protein such that cells partitioned into a given population have a reporter signal within a desired range of reporter signal intensity;

modifying a rate of protein expression or degradation by cells for a given population of cells; and

selecting a subpopulation of the cells from the given population of cells based on whether the cells have different normalized reporter signal intensities than other cells in the given population, the normalized reporter signal intensity comprising a reporter signal from the fusion protein normalized relative to a reporter signal from the first reporter protein, the difference being indicative of the subpopulation of cells expressing shorter lived fusion proteins than the fusion proteins expressed by the other cells in the given population.

33. A method according to claim 32 wherein the method further comprises determining the nucleic acid sequences of the fusion proteins of the selected subpopulation of cells.

34. A method according to claim 32 wherein the method further comprises determining the protein sequences of the fusion proteins of the selected subpopulation of cells.

35. A method for selecting cells based on whether the cells express a short-lived protein, the method comprising:

forming a construct library encoding a library of fusion proteins, each fusion protein comprising a reporter protein and a protein encoded by a sequence from a

5 cDNA library derived from a sample of cells;
6 transducing or transfecting the construct library into cells to form a library of
7 cells which express the library of the fusion proteins;
8 screening the transduced or transfected cells for cells which express the
9 fusion protein;
10 partitioning the screened cells into populations of cells based on an intensity
11 of a reporter signal from the fusion protein such that cells partitioned into a given
12 population have a reporter signal within a desired range of reporter signal intensity;
13 modifying a rate of protein expression or degradation by cells in the given
14 population; and
15 selecting a subpopulation of the cells from the given population of cells
16 based on whether the cells have different reporter signal intensities than other cells
17 in the given population, the difference being indicative of the subpopulation of cells
18 expressing shorter lived fusion proteins than the fusion proteins expressed by the
19 other cells in the given population.

1 36. A method according to claim 35 wherein the method further comprises
2 determining the nucleic acid sequences of the fusion proteins of the selected
3 subpopulation of cells.

1 37. A method according to claim 35 wherein the method further comprises
2 determining the protein sequences of the fusion proteins of the selected
3 subpopulation of cells.

1 38. A method according to claim 35 wherein the library of cells further express
2 an internal standard protein having a different reporter signal than the reporter
3 protein, selecting the subpopulation of cells comprising normalizing the reporter
4 signal from the fusion protein using the reporter signal from the internal standard
5 protein.

1 39. A method according to claim 35 wherein screening the transduced or
2 transfected cells for cells which express the fusion protein is based on detection of
3 the reporter protein.

1 40. A method according to claim 35 wherein screening is performed using a flow
2 cytometer.

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